Claims

1. A method for preparing a solution of a refolded, recombinantly expressed or chemically synthesized eukaryotic membrane protein in monodisperse form, comprising the steps of:

(a) providing membrane protein solubilized in a first detergent,

(b) inducing refolding of said membrane protein into its native or active form, and

(c) performing a size exclusion chromatography on said solution of refolded membrane protein.

2. The method of claim 1, comprising between steps (a) and (b) the further step of:

(a') adding a lipid to said membrane protein solution.

3. The method of claim 1, wherein step (b) comprises the step of:

(b') exchanging said first detergent for a second detergent.

4. The method of claim 1, wherein step (b) comprises the step of:

(b'') diluting said first detergent to an adequately low concentration.

5. The method of claim 1, wherein said membrane protein is selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins as well as partial sequences, homologous sequences, mutated sequences and derived sequences of aforementioned group members.

6. The method of claim 1, wherein said membrane protein is a mammalian, preferably a human protein.

7. The method of claim 1, wherein said membrane protein is a histidine-tagged fusion protein.

8. The method of claim 1, wherein said membrane protein is provided in form of inclusion bodies, preferably as a bacterial expressed protein, more preferably as an *E. coli* expressed protein.

9. The method of claim 1, wherein said membrane protein is provided in form of inclusion bodies being synthesized by means of a cell-free expression system, preferably of the Rapid Translation System (RTS).

10. The method of claim 2, wherein said lipid is selected from the group consisting of: naturally extracted phospholipids and synthetic phospholipids; especially brain polar lipid extract, phosphatidyl choline, phosphatidyl ethanolamine,

cholesterol, phospholipid, ergosterol, asolectin, sphingomyelin, DOPA.

11. The method of claim 2, wherein said lipid is added to a final concentration of about 0,01 to 5 mg/ml, preferably of about 0,05 to 2 mg/ml, more preferably of about 1 mg/ml.

12. The method of claim 1, wherein said first detergent is selected from the group consisting of: FOS-choline-8, FOS-choline-9, FOS-choline-10, FOS-choline-11, FOS-choline-12, FOS-choline-13, FOS-choline-14, FOS-choline-15, FOS-choline-16, and N-laroyl-sarcosine.

13. The method of claim 1, wherein said first detergent is provided in a final concentration of about 0,1 to 5, preferably of about 0,5 to 4, more preferably of about 1 % (w/v).

14. The method of claim 1, wherein in step (b) additionally SDS and/or urea is added to the membrane protein solution.

15. The method of claim 3, wherein said second detergent is selected from the group consisting of charged and uncharged detergents, preferably from the group consisting of: maltosides; alkyl phosphocholines having a chain length of C8 to C16; bile acids and derivatives; alkyl-N,N-dimethyl glycine (alkyl=C8 to C16); alkyl glycosides (alkyl=C5 to C12); glucamides; saccharide fatty acid est rs.

16. The method of claim 3, wherein said second detergent is provided in a final concentration of about 0,01 to 5, preferably of about 0,05 to 1, more preferably of about 0,1 % (w/v).

17. The method of claim 3, wherein in step (b') said exchange is carried out via a chromographic method, preferably via the use of a nickel-NTA column, and/or of a ion exchange column, and/or of an affinity column, and/or of a metal chelate column.

18. The method of claim 3, wherein within step (c) said second detergent is exchanged for a third detergent.

19. The method of claim 18, wherein said exchange is carried out via a chromatographic method, preferably via the use of a nickel-NTA column, and/or of an ion exchange column, and/or of an affinity column, and/or of a metal chelate column, and/or a Superdex 200 column.

20. The method of claim 19, wherein said third detergent is selected from the group consisting of: maltosides; alkyl phosphocholines having a chain length of C8 to C16; bile acids and derivatives; alkyl-N,N-dimethyl glycine (alkyl=C8 to C16); alkyl glycosides (alkyl=C5 to C12); glucamides; saccharide fatty acid esters.

21. A method for preparing a crystalline form of a recombinantly expressed, or chemically synthesized eukaryotic membrane protein, preferably selected from the group consisting of: rec ptors, preferably from the family of

G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences, recombinant forms of aforementioned group members; comprising the steps of:

(a) Providing a solution of said membrane protein in monodisperse form, and

(b) Incubating the solution for growing of membrane protein crystals,

wherein step (a) is performed according to the method of claim 1.

22. A method for preparing a crystalline form of a recombinantly expressed, or chemically synthesized eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences, recombinant forms of aforementioned group members; comprising the steps of:

(a) Providing a solution of said membrane protein in monodisperse form, and

(b) Incubating the solution for growing of membrane protein crystals,

wherein step (a) is performed according to the method of claim 2.

23. A method for preparing a crystalline form of a recombinantly expressed, or chemically synthesized eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences, recombinant forms of aforementioned group members; comprising the steps of:

(a) Providing a solution of a membrane protein in monodisperse form, and

(b) Incubating the solution for growing of membrane protein crystals,

wherein step (a) is performed according to the method of claim 3.

24. A method for preparing a crystalline form of a recombinantly expressed, or chemically synthesized eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences, recombinant forms of aforementioned group members; comprising the steps of:

(a) Providing a solution of a membrane protein in monodisperse form, and

(b) Incubating the solution for growing of membrane protein crystals,

wherein step (a) is performed according to the method of claim 4.

25. The method of claim 21, wherein transition from step (a) to step (b) occurs without interposition of a separation step for separating of protein folded into its native or active form, from protein not folded into its native or active form.

26. The method of claim 22, wherein transition from step (a) to step (b) occurs without interposition of a separation step for separating of protein folded into its native or active form, from protein not folded into its native or active form.

27. The method of claim 23, wherein transition from step (a) to step (b) occurs without interposition of a separation step for separating of protein folded into its native or active form, from protein not folded into its native or active form.

28. The method of claim 24, wherein transition from step (a) to step (b) occurs without interposition of a separation step for separating of protein folded into its native or active form, from protein not folded into its native or active form.

29. The method of claim 21, wherein in step (a) an accessory agent is added to said solution, preferably said agent is selected from the group consisting of: proteins including ligands of membrane receptors, receptors, peptides, antibodies, haptens; nucleic acids including aptamers; organic compounds including ligands of membrane receptors, lipids, sugars; anorganic compounds; drugs; prodrugs.

30. The method of claim 22, wherein in step (a) an accessory agent is added to said solution, preferably said agent is selected from the group consisting of: proteins including ligands of membrane receptors, receptors, peptides, antibodies, haptens; nucleic acids including aptamers; organic compounds including ligands of membrane receptors, lipids, sugars; anorganic compounds; drugs; prodrugs.

31. The method of claim 23, wherein in step (a) an accessory agent is added to said solution, preferably said agent is selected from the group consisting of: proteins including ligands of membrane receptors, receptors, peptides, antibodies, haptens; nucleic acids including aptamers; organic compounds including ligands of membrane receptors, lipids, sugars; anorganic compounds; drugs; prodrugs.

32. The method of claim 24, wherein in step (a) an accessory agent is added to said solution, preferably said agent is selected from the group consisting of: proteins including ligands of membrane receptors, receptors, peptides, antibodies, haptens; nucleic acids including aptamers; organic compounds including ligands of membrane receptors, lipids, sugars; anorganic compounds; drugs; prodrugs.

33. The method of claim 21, wherein step (b) is performed according to standard crystallization screenings by "hanging drop" or/and "sitting drop" vapor diffusion, or/and micro batch, or/and micro dialysis, or/and free interface diffusion technique, said standard crystallization screenings are preferably selected from the group consisting of: Hampton Research Crystal screens, Molecular Dimensions screens, Emerald Biostructures screens, and Jena BioScience screens.

34. The method of claim 22, wherein step (b) is performed according to standard crystallization screenings by "hanging drop" or/and "sitting drop" vapor diffusion, or/and micro batch, or/and micro dialysis, or/and free interface diffusion technique, said standard crystallization screenings are preferably selected from the group consisting of: Hampton Research Crystal screens, Molecular Dimensions screens, Emerald Biostructures screens, and Jena BioScience screens.

35. The method of claim 23, wherein step (b) is performed according to standard crystallization screenings by "hanging drop" or/and "sitting drop" vapor diffusion, or/and micro batch, or/and micro dialysis, or/and free interface diffusion technique, said standard crystallization screenings are preferably selected from the group consisting of: Hampton Research Crystal screens, Molecular Dimensions screens, Emerald Biostructures screens, and Jena BioScience screens.

36. The method of claim 24, wherein step (b) is performed according to standard crystallization screenings by "hanging drop" or/and "sitting drop" vapor diffusion, or/and micro batch, or/and micro dialysis, or/and free interface diffusion technique, said standard crystallization screenings are preferably selected from the group consisting of: Hampton Research Crystal screens, Molecular Dimensions screens, Emerald Biostructures screens, and Jena BioScience screens.

37. The method of claim 33, wherein the "sitting" or "hanging drop" consisting of about 200 nl of membrane protein solution having a concentration of about 1-100 mg/ml, preferably 10 mg/ml of protein, and of about 1 nl-10 ml, preferably 200 nl of precipitant solution, and wherein the reservoir containing about 10 µl-100 ml, preferably 100 µl of precipitant solution.

38. The method of claim 34, wherein the "sitting" or "hanging drop" consisting of about 200 nl of membrane protein solution having a concentration of about 1-100 mg/ml, preferably 10 mg/ml of protein, and of about 1 nl-10 ml, preferably 200 nl of precipitant solution, and wherein the reservoir containing about 10 µl-100 ml, preferably 100 µl of precipitant solution.

39. The method of claim 35, wherein the "sitting" or "hanging drop" consisting of about 200 nl of membrane protein solution having a concentration of about 1-100 mg/ml, preferably 10 mg/ml of protein, and of about 1 nl-10 ml, preferably 200 nl of precipitant solution, and wherein the reser-

voir containing about 10 µl-100 ml, preferably 100 µl of precipitant solution.

40. The method of claim 36, wherein the "sitting" or "hanging drop" consisting of about 200 nl of membrane protein solution having a concentration of about 1-100 mg/ml, preferably 10 mg/ml of protein, and of about 1 nl-10 ml, preferably 200 nl of precipitant solution, and wherein the reservoir containing about 10 µl-100 ml, preferably 100 µl of precipitant solution.

41. The method of claim 37, wherein said precipitant solution has a pH value of about pH 6,5-10 and comprises about 0-0,5 M, preferably 0,1 M Tris/HCl and/or Hepes/NaOH and/or NaK phosphate at that given pH value; about 5-40 % (w/v) of a polyethylene glycol (PEG) and/or polyethylene glycol mono methylether (PEG MME) with a molecular weight of about 1.000-10.000, preferably 2.000-6.000, more preferably 4.000.

42. The method of claim 38, wherein said precipitant solution has a pH value of about pH 6,5-10 and comprises about 0-0,5 M, preferably 0,1 M Tris/HCl and/or Hepes/NaOH and/or NaK phosphate at that given pH value; about 5-40 % (w/v) of a polyethylene glycol (PEG) and/or polyethylene glycol mono methylether (PEG MME) with a molecular weight of about 1.000-10.000, preferably 2.000-6.000, more preferably 4.000.

43. The method of claim 39, wherein said precipitant solution has a pH value of about pH 6,5-10 and comprises about

0-0,5 M, preferably 0,1 M Tris/HCl and/or Hepes/NaOH and/or NaK phosphate at that given pH value; about 5-40 % (w/v) of a polyethylene glycol (PEG) and/or polyethylene glycol mono methylether (PEG MME) with a molecular weight of about 1.000-10.000, preferably 2.000-6.000, more preferably 4.000.

44. The method of claim 40, wherein said precipitant solution has a pH value of about pH 6,5-10 and comprises about 0-0,5 M, preferably 0,1 M Tris/HCl and/or Hepes/NaOH and/or NaK phosphate at that given pH value; about 5-40 % (w/v) of a polyethylene glycol (PEG) and/or polyethylene glycol mono methylether (PEG MME) with a molecular weight of about 1.000-10.000, preferably 2.000-6.000, more preferably 4.000.

45. A crystalline form of a recombinantly expressed or chemically synthesized, eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences of aforementioned group members.

46. A crystalline form of a recombinantly expressed or chemically synthesized, eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences of aforementioned group members; wherein

said crystallized membrane protein is prepared according to the method of claim 21.

47. A crystalline form of a recombinantly expressed, or chemically synthesized eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences of aforementioned group members; wherein said crystallized membrane protein is prepared according to the method of claim 22.

48. A crystalline form of a recombinantly expressed, or chemically synthesized eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences of aforementioned group members; wherein said crystallized membrane protein is prepared according to the method of claim 23.

49. A crystalline form of a recombinantly expressed, or chemically synthesized eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences of aforementioned group members; wherein

said crystallized membrane protein is prepared according to the method of claim 24.

50. A crystalline form of a complex of a recombinantly expressed, or chemically synthesized eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences of aforementioned group members, and of an accessory agent.

51. A crystalline form of a complex of a recombinantly expressed, or chemically synthesized eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences of aforementioned group members, and of an accessory agent, wherein said complex is prepared according to the method of claim 29.

52. A crystalline form of a complex of a recombinantly expressed, or chemically synthesized eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences of aforementioned group members, and of an accessory agent, wherein said complex is prepared according to the method of claim 30.

53. A crystalline form of a complex of a recombinantly expressed, or chemically synthesized eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences of aforementioned group members, and of an accessory agent, wherein said complex is prepared according to the method of claim 31.

54. A crystalline form of a complex of a recombinantly expressed, or chemically synthesized eukaryotic membrane protein, preferably selected from the group consisting of: receptors, preferably from the family of G-protein-coupled receptors, ion channels, transport proteins, as well as partial sequences, homologous sequences, mutated sequences and derived sequences of aforementioned group members, and of an accessory agent, wherein said complex is prepared according to the method of claim 32.